Abiotic Factors, Particularly CO_2 concentration, Affecting Carnivorous Plants from the Eastern Shore of Maryland

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Abstract

Carnivorous plants, particularly bladderworts (*Utricularia* spp.; Lentibulariaceae), have long been known to grow on the Eastern Shore of Maryland, but few if any studies have examined their growth *in situ* or in the laboratory along with the abiotic factors which affect their growth. From the scientific literature, Great Swamp in Maryland (Kent County) clearly stands as an unusual site for the Eastern Shore of Maryland, containing several species of bladderworts. Upon investigation, four species of aquatic bladderworts were identified. Several sites within Great Swamp, containing or lacking plants of *Utricularia* spp., were routinely sampled to seek correlations between abiotic components of the local aquatic environment, such as pH and [CO_2], and the presence of bladderworts. Small samples of various *Utricularia* spp. were subjected to a variety of experimental treatments in the laboratory to examine further the factors identified in Great Swamp. Moderate irradiance levels and elevated concentrations of dissolved CO_2 both promoted the growth of *U. intermedia* and *U. macrorhiza*.

Introduction

Carnivorous plants have been an attraction for both botanists and horticulturists since before the time of Charles Darwin. These plants usually grow in nutrient-poor environments, thriving in conditions which many species find daunting. The occurrence of carnivorous plants in these environments relates to their special traits, since to be considered carnivorous species must lure, trap, and digest prey to obtain scarce nutrients especially nitrogen (D'Amato, 1998). Factors besides the availability of organic nitrogen may influence the growth of particular carnivorous plants in particular environments. For example, just as irradiance levels may dictate the growth of understory plants versus canopy species, some biotic factors such as the levels of inorganic nutrients or of pH might help to determine the presence or absence of certain carnivorous plants.

Among carnivorous plants growing in the Eastern United States are the American pitcher plants (*Sarracenia* spp., Sarraceniaceae); the sundews (*Drosera* spp., Droseraceae); the Venus Flytrap (*Dionaea muscipula*, Droseraceae); the butterworts (*Pinguicula* spp., Lentibulariaceae); and the bladderworts (*Utricularia* spp.; Lentibulariaceae). Bladderworts can be found growing as terrestrial, subaquatic, affixed aquatic, or submersed (suspended) aquatic plants (Taylor, 1989).

As indicated in the works of Tatnall (1946) and Sipple (1999), *Utricularia* spp. seem to be the most common carnivorous plants found on the Eastern Shore of Maryland, and, in particular, Sipple (1999) points to Great Swamp in Kent County, Maryland, as a site containing an unusual abundance of bladderworts. This site (which is actually a bog) consists of several interlinked channels of water created by the mining of sphagnum peat. A portion of this area privately owned and under protection by The Nature Conservancy, Echo Hill Camp and Echo Hill Outdoor School, permitted access to part of Great Swamp.

Several sites were selected for routine measurements of water quality and for observations of bladderworts (see Table 1). These sites varied with regard to the amount of irradiance received, location near the bank or in mid-channel, and the number of species which the sites contained. These sites were tested for a variety of abiotic factors, both chemical factors such as phosphate levels and physical factors such as the amount of ambient irradiance, to determine any relationship to the presence or absence of bladderworts.

In previous studies from other geographic regions, communities of bladderworts have been shown to exist under a wide range of temperatures and nutrient conditions (Roberts, *et al.*, 1985; Adamec & Lev, 2001). It has been shown by previous research that nitrogen and phosphorous levels have a significant impact upon the growth of bladderwort communities (Havens *et al.*, 1999).

Non-destructive laboratory experiments were also conducted on small samples of bladderworts from Great Swamp to further explore connections between levels of illumination or dissolved carbon dioxide and the growth of bladderworts. Previously, Adamec (1999) showed, and others confirmed (Camilleri, 1999) that elevated concentrations of CO_2 in the aquatic environment accelerate the growth of *Aldrovanda vesiculosa* (Droseraceae). This is another aquatic carnivorous plant, native to Europe, Asia, Africa, and Australia, so levels of CO_2 in Great Swamp might influence growth of bladderworts there.

Site #	Description
1	Mid-channel site next to fallen tree, bladderworts present among *Nymphaea odorata*.
2	Bank site under heavy canopy. Largest community of bladderworts seen, also large amounts of hornworts were observed.
3	Center of channel, no bladderworts present.
4	Bank site under heavy cover, receives afternoon sun. Bladderworts pre sent.
5	Bank site with no cover, receives afternoon sun. Small numbers of bladderworts present.
6	Bank site next to birdhouse, receives direct morning sunlight. Bladderworts present among water lilies but not in nearby open water.
7	Bank site receiving evening sunlight, with heavy cover. No bladderworts present.

Table 1: Description of Sites in Great Swamp for Water Sampling

Methods

Using Taylor (1989), a monograph on the genus *Utricularia* currently accepted as the standard taxonomic text, samples of *Utricularia* spp. from Great Swamp were identified.

Water samples were taken from several sites in Great Swamp, described in Table 1, weekly for six weeks during June, July, and August of 2000. These samples were tested as described in the LaMotte Monitors Handbook (Campbell, 1992). Tests were performed for dissolved CO_2 (test kit #7297), toxic ammonia (#59100), salinity (#7459), copper content (#10269), pH (#5090), nitrates (#3110), and phosphate (#3114). New 50 ml polyethylene sample vials were used for each sample collection. At the pHs measured, all ammonia would have been in the form of NH_4^+, and the data presented are for the appropriate test strip portion (Freshwater Aqualab IV Mardel Glendale Heights IL).

For all experiments in the laboratory, clear plastic containers were used as aquaria to contain bladderworts, and all replicates of a given experiment were conducted in identical containers. Plants were grown in a laboratory in which the temperature ranged from 20-25°C, approximately consistent with the range of aquatic temperatures in which the bladderworts were found growing in Great Swamp.

To observe the effects of varying levels of irradiance, plants of *U. intermedia*, *U. macrorhiza*, and *U. gibba* were grown individually in aquaria (three in parallel per repetition; approximately 500 ml of doubly distilled H_20 per aquarium to start) and placed under constant irradiance provided by cool white-type fluorescent bulbs. Controls received full strength irradiance (200-300 lux) while experimental plants were grown with the same placement relative to overhead lights, but their aquaria were covered by two layers of fiberglass window screen to decrease illumination (60-80 lux). The experiment was run for three weeks per repetition for three repetitions, and measurements were taken weekly of plant length, fresh weight, number of traps produced, and the amount of irradiance received. The fresh weight of the plants was measured after blotting excess water, starting with one plant per aquarium or the same initial fresh weight if plants were small in the case of *U. geminiscapa* in some experiments. Irradiance measurements were made using a Fisher Scientific Dual Range Light Meter (Fisher Scientific, Pittsburgh, PA, USA).

Experiments were also conducted to determine the effect of varying levels of CO_2 on the growth of bladderworts from Great Swamp. Controls were placed individually in separate containers and treated as for varied levels of illumination, using the same species as in those experiments. Experimental plants were treated identically to controls except that CO_2 generators assembled according to Camilleri (1999) were used to constantly bubble CO_2 through their aquaria. The experiment was repeated three times for three weeks per repetition, and measurements were taken weekly of plant length, the number of traps produced, fresh weight, and CO_2 levels.

Results

Three species of submersed aquatic bladderworts and one affixed species grew in Great Swamp: *U. gibba* (submersed), *U. geminiscapa* (submersed), *U. macrorhiza* (submersed); *U. intermedia* (affixed). *Utricularia intermedia*, *U. macrorhiza*, and *U. gibba* had been found previously in Great Swamp by Sipple (1999). These plants were identified mainly by leaf shape and bladder shape, and identification was confirmed for *U. macrorhiza* and *U. gibba* by scape characteristics. These were the only two species to flower during the period of observations.

Taylor (1989) clearly states that in spite of the similarities between the European *U. vulgaris* and the American *U. macrorhiza*, they are to be referred to separately, so his authority was followed. *Utricularia gibba* and *U. macrorhiza* formed the great majority of plants found in the open swamp, where the seven sites for study were found. *Utricularia intermedia* was found in an area of much narrower and shallower channels along with those two species. Water quality was examined less frequently in areas hosting *U. intermedia*, but in those sites the concentration of carbon dioxide was similar to other sites containing a large number of bladderworts, i.e. 14-20 ppm. *Utricularia geminiscapa* was only found occasionally, in areas of shaded and open water. For laboratory experiments, *U. macrorhiza*, *U. gibba*, and *U. intermedia* were used.

Water analysis at Echo Hill, Maryland, shows that the area of study had consistent levels for most of the abiotic factors examined at most sites studied during the six weeks (June-August, 2000) of the study: pH (6.0; mean for all sites for 6 weekly measurements), nitrate (0.43-0.46 ppm), phosphate (0.25-0.29 ppm), salinity (1.02 ppt), copper (0.25-0.42 ppm), and temperature (24.4-24.7°C). Temperatures were always within the range 23-26°C, even for the most extreme measurements during this period. Levels of CO_2 and ammonia did vary among sites as shown in Figure 1.

Stands of *U. intermedia*, *U. gibba*, and *U. geminiscapa* were found growing under heavy to moderate cover along a boardwalk built through the swamp, where the irradiance level varied from 2100-2200 lux from bright overcast to sunny conditions. Similar levels of illumination were found near the banks of channels. Both *U. macrorhiza* and *U. gibba* were found growing in open water in the channels of the swamp, though almost all stands of bladderworts observed were found with at least partial shading by nearby aquatic non-carnivorous species. The irradiance level in those areas and in areas with no floating cover, in which only a few plants of *U. macrorhiza* and *U. gibba* could be found, varied from 20,000-21,000 lux.

All data from the growth experiments were normalized by dividing measurements for both control and experimental plants by the initial value for the control plant in a given repetition. In Figures 2-3, data are shown without error bars because the variation in the size of the plants which were available to start

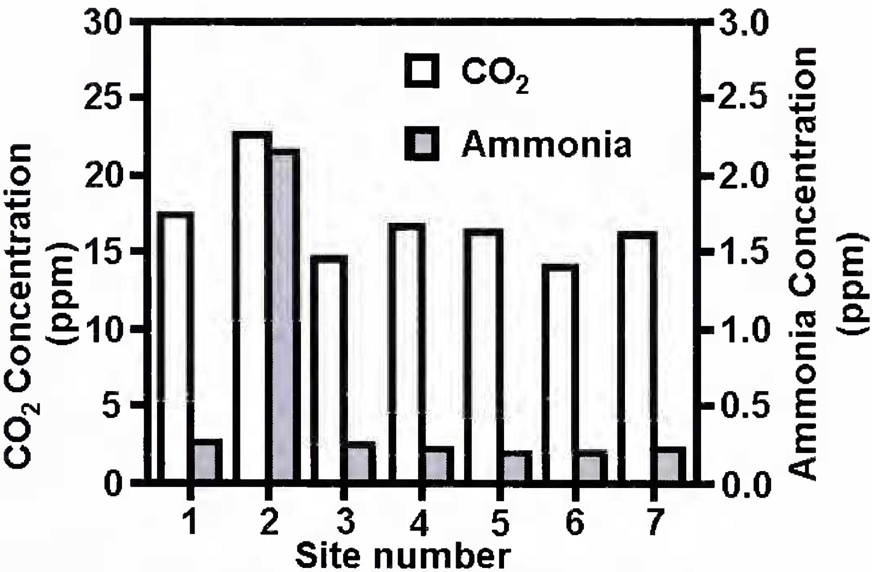

Figure 1: Levels of ammonia and dissolved carbon dioxide at seven sites in Great Swamp. Mean±SD for six weekly measurements (not plotted) is 2.8 ppm for CO_2 and 0.73 ppm for ammonia. For descriptions of sites, see Table 1.

experiments was large. This introduces a large variation when experimental results from separate repetitions are averaged, even after normalization. In spite of this, the trends seen in Figures 2-3 for various experiments can also be seen when examining data from individual experiments.

When the level of illumination was varied in the laboratory, irradiance levels for the control plants were around 200-300 lux and experimental irradiance levels were at 60-80 lux. Controls of *U. intermedia* and *U. macrorhiza* both showed weaker growth in length with reduced irradiance levels. (Figure 2a,b). *Utricularia intermedia* also showed a rather large increase in the amount of traps produced at the control irradiance levels (Figure 2c). *U. gibba* growth was unchanged by changing irradiance levels (data not shown).

When the level of CO_2 was varied, levels for experimental plants rose to around 13 ppm, compared to control levels at around 3 ppm. Elevating levels of CO_2 enhanced growth of *U. macrorhiza* and *U. intermedia*, as exemplified in Figure 3, displaying increases in trap number and fresh weight for *U. intermedia*.

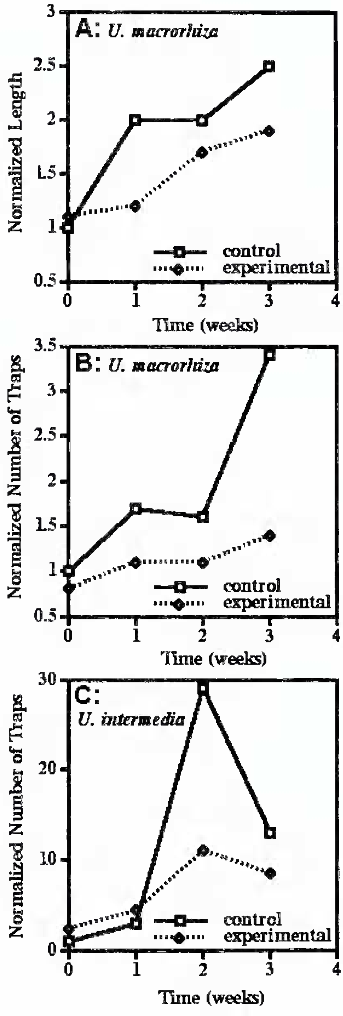

Figure 2: Effect of varied irradiance level (control plants approximately 200-300 lux; experimental approximately 60-80 lux) on the growth of bladderworts in the laboratory. A. Change in length of *U. macrorhiza* over three weeks. B. Change in number of traps for *U. macrorhiza*. C. Change in number of traps for *U. intermedia*. Mean of three repetitions. Normalization and the lack of errors bars are discussed in the text.

Discussion

The trends observed in the water quality analysis fit what is expected for a site containing bladderworts. Bladderworts can be found growing in water within a pH range of 4-8 (Roberts *et al.*, 1985; Adamec & Lev, 2001), and in waters with relatively low levels of nitrogen and phosphorus (Havens *et al.*, 1999), as were found at Great Swamp where pH was typically 6 and nitrogen and phosphorus were both less than 0.50 ppm. Miniscule amounts of copper and ammonia were observed, but as to whether or not they had an effect on the growth of bladderworts is unknown and is a subject for future testing. Carnivorous plants most commonly grow in acidic environments, and this was true for Great Swamp. The levels of ammonia and phosphorus which were measured were near or just below the recommended limits for the testing methods used, so the principal point taken is that Great Swamp is an area of moderate abundance for these nutrients.

Stands of bladderworts were most often observed growing in moderately to heavily shaded areas, most often crowded among *Nymphaea odorata* (Nymphaceae), hornwort (*Ceratophyllum demersum*, Ceratophyllaceae), and *Nuphar advena* (Nymphaceae). Levels of irradiance in the shade were not fully replicated in the laboratory by control treatments, but plants grown in the laboratory at levels closest to those preferred in nature showed increases in both plant length and trap production compared to plants treated with lower levels of irradiance (see Figure 2).

Bladderworts in Great Swamp probably prefer irradiance levels lower than those of full sunlight, but very strong shade, represented by the experimental irradiance treatment used in the laboratory, would be inhibitory of their growth. Even those few plants which were found in full sun in open water grew among other non-carnivorous aquatic plants which provided ample local shade. Production of irradiance intensities in the laboratory closer or equal to those of full sun should be examined in future, using a method which offsets heat production to avoid confounding experimental results. Higher levels of irradiance would also help to resolve any concerns about the levels used here having been at or below the compensation point for photosynthesis. (Although it should be noted that the laboratory plants did grow actively.)

Bladderworts growing both in the wild and in the laboratory at these irradiance levels maintained a vibrant green color, while plants in the laboratory and in the wild turned a reddish-yellow when subjected to higher irradiance levels. At Echo Hill, *U. macrorhiza* was observed as the species most tolerant to a range of irradiance levels, as it could be found both in full shade crowded among other plants or in full sunlight. However, in full sunlight it was usually found growing among other aquatics and was probably not exposed to full sunlight. *Utricularia gibba* was also observed in both areas, while *U. intermedia* only occurred in shallow and shaded sites.

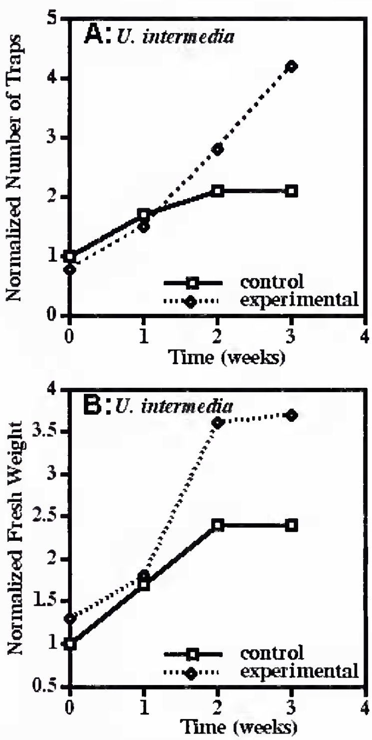

Figure 3: Effect of varied levels of CO_2 on the growth of *U. intermedia*. A: Change in number of traps. B: Change in fresh weight. Control level of CO_2 was approximately 3 ppm; experimental level was approximately 13 ppm. Mean of three repetitions. Normalization is explained in the text.

Utricularia gibba and *U. macrorhiza* in full sun grew slightly submerged and did not form partially-exposed surface mats. Such mats did occur in the shade, however. Inflorescences of both of these plants were observed during the first three weeks of July (*U. macrorhiza*), and during the last week in July and the first week in August (*U. gibba*), but only along bank sites that received partial to full shade.

Levels of CO_2 in the wild were higher than those achieved in the laboratory using a CO_2 generator. However, the strongest growth of plants in the wild occurred at sites 1 and 2, where the highest levels of CO_2, significantly higher than other sites in the case of site 2, were recorded (see Figure 1). However, levels in the laboratory may have been depressed by strong absorption of CO_2 by the rapidly growing plants, or because the temperatures was higher in the laboratory. It was observed that *U. intermedia*, like *Aldrovanda vesiculosa*, showed increased growth in length and trap production due to increased CO_2 levels (see Figure 3), probably due to the use of CO_2 in photosynthesis (Adamec, 1999). The trends in

Figure 4: *Utricularia* habitat. Photo by Matthew McDermott.

growth, comparing control and experimental plants, were visible in the replicates performed for each experiment, even though variations in the size of starting plants made statistical analysis difficult.

Both *U. macrorhiza* and *U. gibba* seemed insensitive to CO_2 levels alone. Perhaps both irradiance and CO_2 levels are important for the growth of bladderworts. *U. gibba*, the most widespread bladderwort in the world (D'Amato, 1998) and perhaps the most adaptable, may not be affected strongly by the CO_2 levels used in this study, but irradiance levels do seem important based on the placement of this plant. Field observations by one author (DWD) of *U gibba* and *U. muelleri*, another floating bladderwort, in the Northern Territory of Australia indicate a similar preference there.

The distribution of traps and comparisons of trap production in response to biotic versus abiotic factors were not attempted in this pilot study. However, such work should be conducted in future in view of two papers: Knight & Frost (1991) demonstrated that *U. macrorhiza* may change the numbers of bladders in response to abiotic factors, while Friday (1989) showed that trap age and position are crucial for evaluating the growth responses of bladderworts. Furthermore, Richardson (2001) has recently suggested with a study of *U. purpurea* that bladders may also have non-carnivorous importance in their functional ecology *viz.* a mutualistic relationship with microinvertebrates surviving in the traps.

Utricularia macrorhiza and *U. intermedia* seem to be affected by both irradiance and the concentration of CO_2, and the different responses of these plants to elevated concentrations of CO_2 might have explanations related to growth patterns—*e.g. U. macrorhiza* may grow to greater lengths at first to allow its modified stems to photosynthesize more. Increased trap production may be more important for the normally short *U. intermedia*, which seems to grow only in shade, than for *U. macrorhiza*. Such factors, with the addition of biotic factors such as prey availability, will be examined in future summers.

In future studies tests for potassium, calcium, and magnesium should be added to the suite of tests used in this study as they have been shown to have an impact upon the occurrence of *U. intermedia* and *U. ochroleuca* at sites studied in the Czech Republic (Adamec & Lev, 2001). The experiments performed during the period considered here did not allow for correlation between levels of some abiotic factors, such as ionic concentrations, and distributions of bladderwort species. This may be due in part to the nature of the tests used, and further experiments to explore such variables are planned for summer 2001. Measurement of photosynthetically active radiation using radiometric units will then be possible as well, due to recent acquisition of equipment, and a broader range of CO_2 concentrations should also elucidate the requirements of these species.

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